



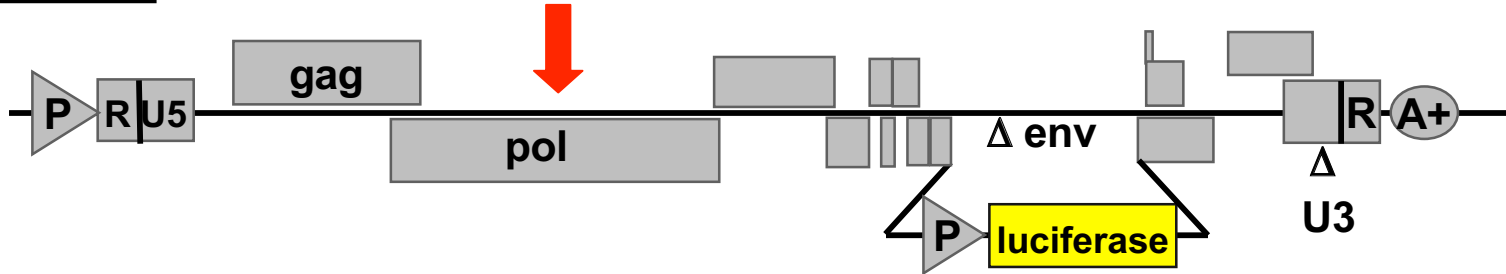
# **Standardization of HIV Neutralization Assays NeutNet**

**Villa Cagnola  
March 17-18, 2007**

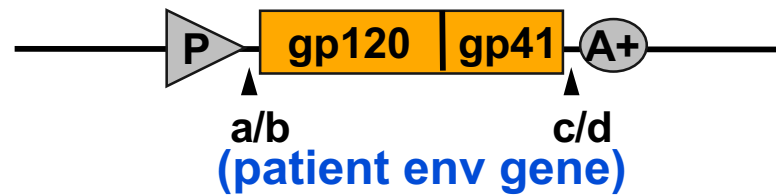
# Monogram Neutralization Assay

## Vectors:

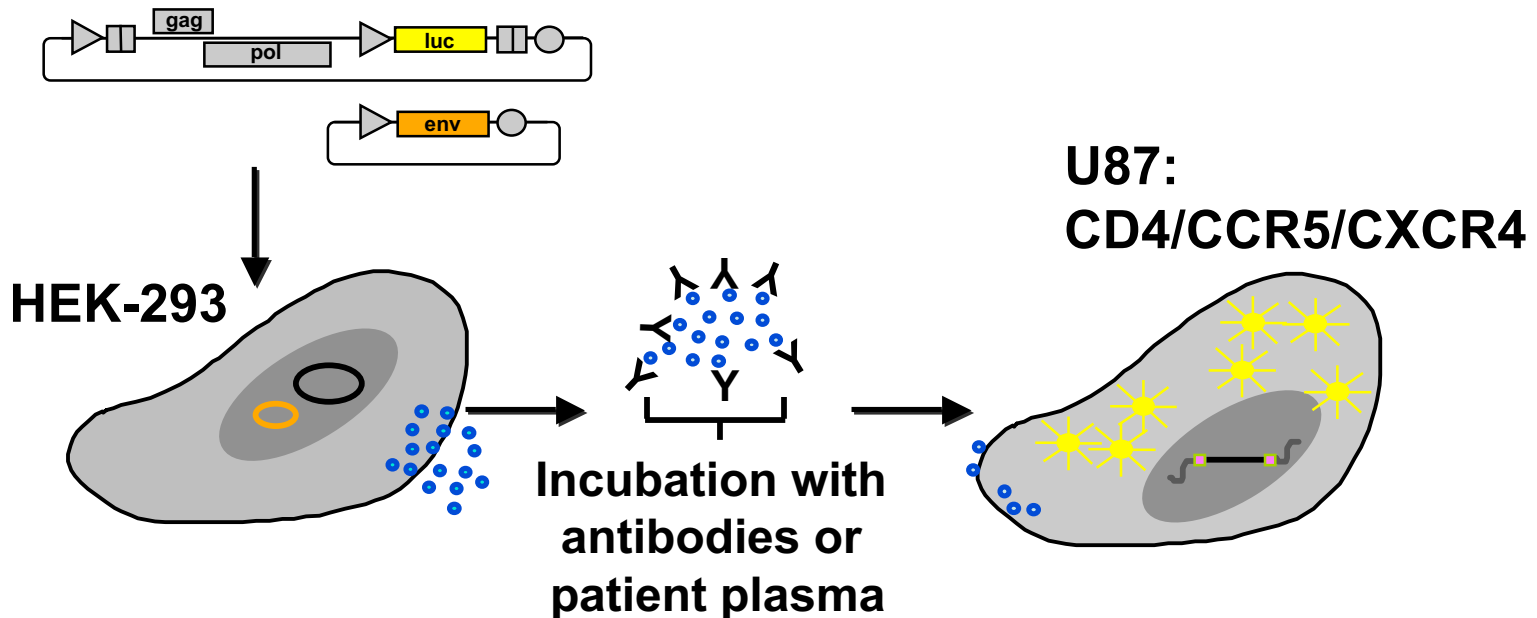
Drug resistant PR/RT can be used for testing plasmas with RT inhibitors



Clone population of viral env present in plasma, cell culture, etc.



## Neutralization Assay:

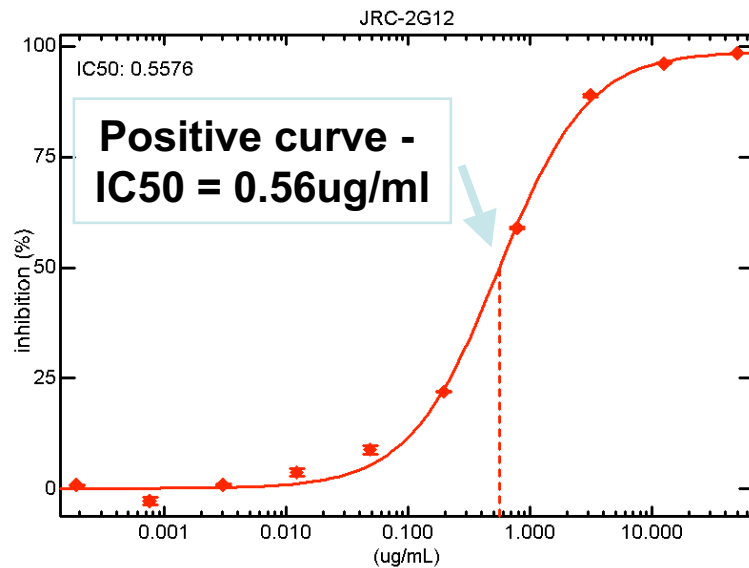


# Neutralization Assay Controls

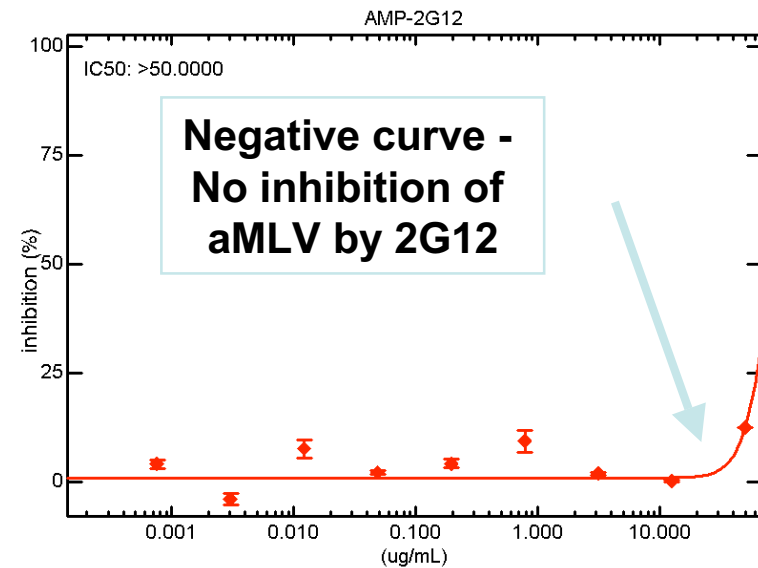
- **Three control viruses tested with every plasma:**
  - NL43 – neutralization sensitive lab strain
  - JRCSF – well known primary isolate
  - aMLV – specificity control:
    - A test IC50 must **≥3X** IC50 with aMLV to be called positive (green color)
- **HIV+ Plasma Control:**
  - Tested with every virus in every run
  - One control for every 7 sera tested
  - N16, Z23 – Broad neutralizing capability

# Antibody Neutralization Assay Inhibition Curves and Specificity Control

## JRCSF



## aMLV



**aMLV = specificity control**  
**Anti-HIV antibodies will not inhibit aMLV.**

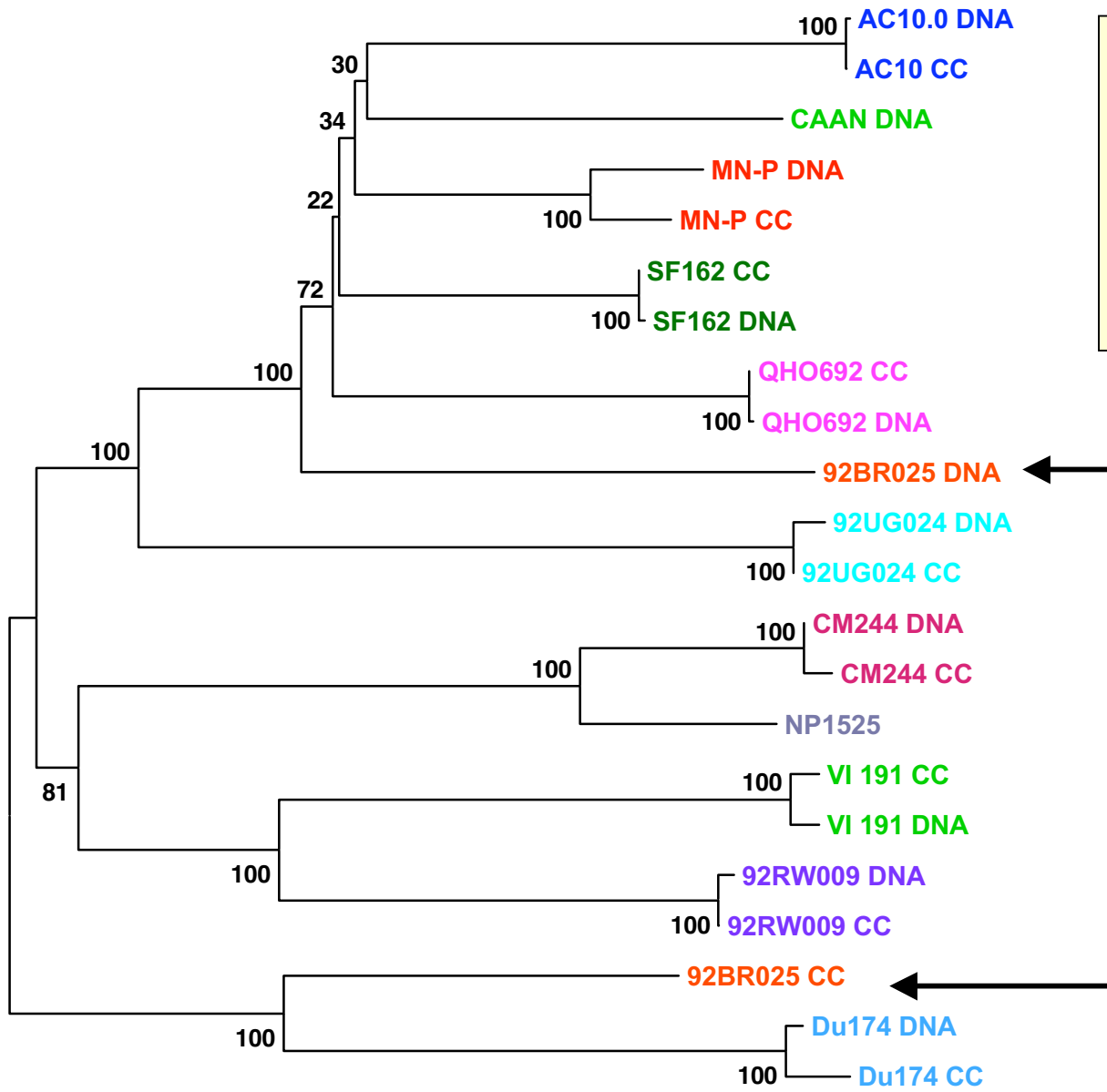
# Neut Net Samples

- Vector construction – cloned pool of envelopes from cell culture sups and re-cloned envelope from vectors into expression vector
- Tropism
- Neutralization
- gp160 sequence

Table 1: Tropism Assay Data

Virus	Subtype	Luciferase Activity (RLU) in U87/CD4/CXCR4 cells			Luciferase Activity (RLU) in U87/CD4/CCR5 cells			Tropism
		No drug	+CXCR4 inhibitor	+CCR5 inhibitor	No drug	+CXCR4 inhibitor	+CCR5 inhibitor	
HXB2	B	247,038	236	268,062	58	57	50	X4
JRC5F	B	133	53	60	377,471	371,428	559	R5
ASM54	B	2,510	57	2,868	39,503	38,237	100	DM
92TH596	B	1,219,078	38,397	1,249,372	431,428	392,638	733	DM
ASM3	B	81	58	61	681,543	624,439	920	R5
93RW018	A	119	69	82	416,319	424,348	783	R5
93RW024	A	1,587,048	1,524	1,539,804	729,679	709,460	1,015	DM
94KE105	C	126	63	58	584,826	670,084	6,788	R5
93UG053	D	3,209,046	9,388	3,456,138	84	118	64	X4
93UG070	D	254,095	330	261,035	64	50	57	X4
CMU02	CRF01_AE	235,656	4,627	228,640	94	52	225	X4

Representative luciferase activity data and tropism calls are shown. As luciferase activity approaches background levels, reduction in RLU in the presence of inhibitors becomes increasingly important in making the tropism call.



gp160 sequences  
prove same  
source for  
matched clones  
and pools except  
92BR025

# NCBI Blast Results

92RW009 CC	OK - identity correct
92RW009 DNA	OK - identity correct
92UG024.2 DNA	OK - identity correct
92UG024 CC	OK - identity correct
AC10 CC	OK - identity correct
AC10.0 cl 29 DNA	OK - identity correct
BR92025 DNA	No match with reference seq
BR92025 CC	OK - identity correct
CAAN5342 DNA	OK - identity correct
CM244 CC	OK - identity correct
CM244 DNA	OK - identity correct
DU174 CC	OK - identity correct
DU174.15 DNA	OK - identity correct
MN-P CC	OK - identity correct
MN-P DNA	OK - identity correct
NP1525 CC	OK - identity correct
QHO692 CC	OK - identity correct
QHO692 cl 42 DNA	OK - identity correct
SF162 CC	OK - identity correct
SF162 DNA	OK - identity correct
VI 191 CC	OK - identity correct
VI 191 DNA	OK - identity correct

Virus	Virus Source	IC50 (ug/ml)			
		4E10	447D-52D	sCD4	TriMab
92RW009	CC	>50	>50	40.347	9.254
92RW009	DNA	>50	>50	>50	32.833
92UG024	CC	4.885	>50	0.573	0.815
92UG024	DNA	10.678	>50	4.143	2.288
AC10	CC	2.972	>50	29.666	5.827
AC10 0 cl 29	DNA	2.338	>50	49.659	4.831
BR92025	CC	18.497	>50	4.581	2.692
BR92025	DNA	5.366	>50	2.624	2.348
CAAN5342	DNA	15.646	>50	45.208	>50
CM244	CC	9.655	>50	7.253	12.037
DU174	CC	22.741	>50	10.231	0.622
DU174	DNA	11.954	>50	4.828	1.646
MN	CC	7.077	0.981	0.561	0.243
MN-P	DNA	1.684	1.180	0.529	0.069
MN VL	Assay Control	0.156	0.010	0.054	0.019
NP1525	CC	2.831	>50	5.126	3.959
QHO 692	CC	18.558	>50	3.838	0.815
QHO692 cl 42	DNA	17.199	>50	3.215	1.112
SF162	CC	18.217	0.233	1.270	0.221
SF162	DNA	21.803	0.177	0.825	0.191
SF162 VL	Assay Control	5.468	0.009	0.131	0.068
VI 191	CC	16.974	1.736	0.172	1.070
VI 191	DNA	15.916	>50	12.766	7.304
JRCSF	Assay Control	7.676	15.749	7.908	0.310
JRCSF	Assay Control	5.653	21.443	15.306	0.521
JRCSF	Assay Control	7.601	13.704	8.850	0.372
NL43	Assay Control	15.458	0.009	0.078	0.515
NL43	Assay Control	7.256	0.005	0.078	0.340
NL43	Assay Control	9.861	0.014	0.129	0.603
aMLV	Assay Control	>50	>50	>50	>50
aMLV	Assay Control	>50	>50	>50	>50
aMLV	Assay Control	>50	>50	>50	>50

**IC50s (ug/ml) of  
Neut Net Isolates  
With MAbs and  
sCD4**

# Difference Between DNA and Cell Culture Version

## Max/Min IC50

	4E10	447D-52D	sCD4	TriMab
92RW009	1.0	1.0	>1.2	3.5
92UG024	2.2	1.0	7.2	2.8
AC10	1.3	1.0	1.7	1.2
BR92025	3.4	1.0	1.7	1.1
DU174	1.9	1.0	2.1	2.6
MN	4.2	1.2	1.1	3.5
QHO692	1.1	1.0	1.2	1.4
SF162	1.2	1.3	1.5	1.2
VI 191	1.1	>28.8	74.3	6.8

>5X difference

# VI191 Cell Culture and DNA clone sequence

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      10      20      30      40      50      60      70      80      90     100
VI 191 CC  MRVKGIQMNWQYLW--TLILGLVIIICNAENLWVTVFYGVPVWRDADTTLFCASDAKAYSEXKHNVWATHACVPTDDPDPKEINLVNVTEDFNMWKNKMVE
VI 191 DNA  .....H..KWG.....G.....K..E.....E.....X.

      110     120     130     140     150     160     170     180     190     200
VI 191 CC  QMQEDIISLWXXSLKPCVKLTPLCVTLXCHNSTTPDNNXTQSTIIDETTNCTYNMTELRDKVQXVHSLFYRLDVVQXNGINGTSNXNGXLYRLINCNTS
VI 191 DNA  .....DQ.....E.....T.....K.....I.....N.SAQ.....

      210     220     230     240     250     260     270     280     290     300
VI 191 CC  XITQACPKVSEFPIPIHYCAPAGFAILKXDKEXNGTGPCXNVSSVQCTHGKXKPVVSTQLLLNGSLAEKIRIRSENFTDNGKNIIVQLVNPVKINCTRP
VI 191 DNA  A.....R..F..R.....I.....M.....T.....E.....

      310     320     330     340     350     360     370     380     390     400
VI 191 CC  SNNTRKGIHIGPGRAIYATGEIIGDIRQAHCNLSRTEWNKTLHQVAIQLREHFITKNTTIIFNSSSSGGGLEITTHSFNCGGEFFYCSTSGLFNSTWENGT
VI 191 DNA  .....F...Q.T.....

      410     420     430     440     450     460     470     480     490     500
VI 191 CC  QVXNDTGSNDTITXXCRIKQIVXMXRVGQAMYAPPIQGXRICESNITGLILTRDGVGNSTNETFRPGGDMRDNRSELYKYVVKIEPLGVAPSPRAK
VI 191 DNA  ..A.....LP.....R.Q.....M.....

      510     520     530     540     550     560     570     580     590     600
VI 191 CC  RRVVAREKRAVGLGAVFLGFLGAAGSTMGAASLTTLTVQARQLLSGIVQQSNLLRAIEAQHLLQLTVWGVIKQLQARVLALERYLRDQQLLGIWGCSSGKL
VI 191 DNA  .....

      610     620     630     640     650     660     670     680     690     700
VI 191 CC  ICPTNVPWNYSWSNKSYYDDIWNNTWLQWDKEIDNYTQLIYGLIEESQNOQEKNEODLLALDKWASLWSWFDISNWLWYIRIFIMIVGGGLIGLR
VI 191 DNA  .....IVFAVL

      710     720     730     740     750     760     770     780     790     800
VI 191 CC
VI 191 DNA  SIINRVRQGYSPLSFQTLPHHQREPDPRPEGIEEGGGEQDRGRSIRLVSGFLALAWDDLRSICLFSYHRLRDFILIVARIVETLGR-----RGWEALKY

      810     820     830     840     850     860
VI 191 CC
VI 191 DNA  LWNLLLYWGRELKISAITLLDATAIAVARWTDRIIEIGQRIVRAILNIPRRIRQGAEKALL*

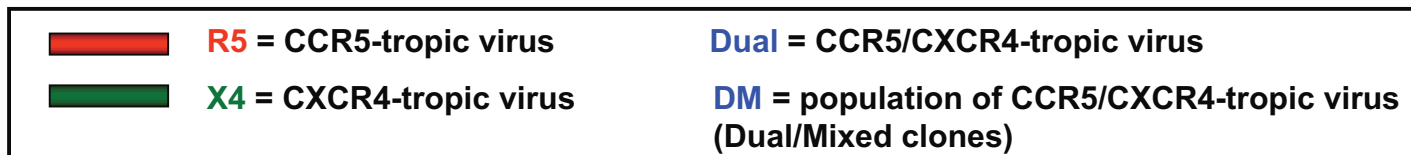
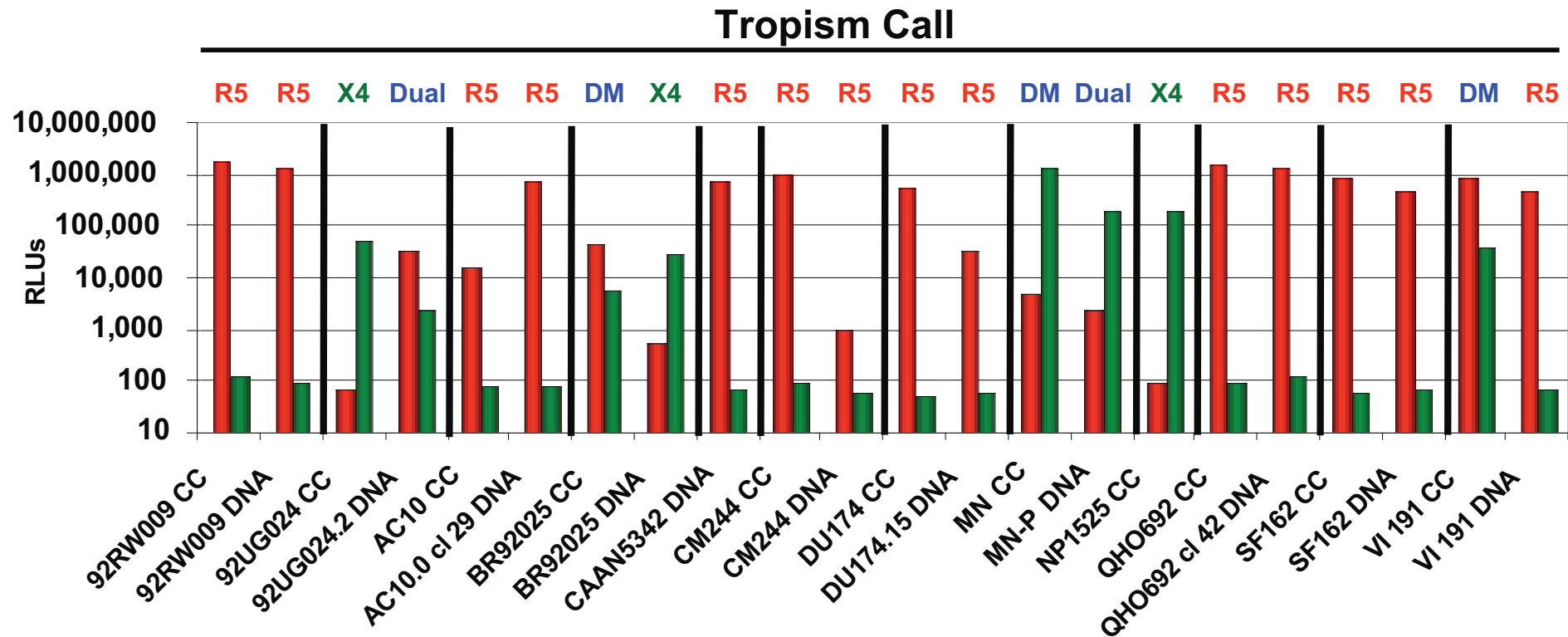
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# 92UG024 Cell Culture and DNA clone sequence

	10	20	30	40	50	60	70	80	90	100
94UG024 CC	MRVREMQRNYQHXXWGIIMLLGMXMCISGTXEQXWVTVYGVVPVWKEATTTLFCASDAKAYKAEAHNIWATHACVPTDENPREIQLENVTEENFMWKNNM									
94UG024 DNA	.....L.K.....L.....E.S.....									
	110	120	130	140	150	160	170	180	190	200
94UG024 CC	VEQMHEDEVISLWDQSLKPCVKLTPLCVTLNCLIEWKNEKETGNKTVDPDKDIGMKNCSFNVTTEVRDKKKQVYALFYKLDVVQMNDNNTXTSYRLINCNT									
94UG024 DNA	.....N.....									
	210	220	230	240	250	260	270	280	290	300
94UG024 CC	SAITQXCPKVTFEPIPIHYCAPAGFAILKCNNKMFNGTGPCNNVSTVQCTHGIRPVVSTQLLLNGSLAEEEEIIIRSENITNNAKIIIVHINESVVINCTR									
94UG024 DNA	.....E.....									
	310	320	330	340	350	360	370	380	390	400
94UG024 CC	PYNINRQRTPIGLGQALYTTTRRIEDIRRAHCNISEAAWKKTLEQVAKKLVLFNXTTIDFKPSSGGDPEITTHSFNCGGEFFYCNTSXXFNHSVGENNTL									
94UG024 DNA	.....R.....KL.....									
	410	420	430	440	450	460	470	480	490	500
94UG024 CC	SIXNXVNXNITLPCIKIQIVNMWQVGVKAMYAPPIQGLIRCSSNITGLLLTRDGGNTSQNEXFRPGGDMRDNRSELYKYKVIKIEPLGLAPTRAKRRV									
94UG024 DNA	..S.G..T.....T.....									
	510	520	530	540	550	560	570	580	590	600
94UG024 CC	VAREKRAIGLGAVFLGFXGAAGSXMGAASLTLTVQARQXMSGIVQQNNLLRAIEAQOXLLQLTVWGIKQLQARVLAVESYLKDQQLLGXWGCGRHICP									
94UG024 DNA	.....L.....T.....L.....H.....V.....									
	610	620	630	640	650	660	670	680	690	700
94UG024 CC	TXVPWNSSWSNKSIDEIWGNMTWMEWEREISNYTGLIYNLIEESQIQQEKNEKXXLELDKWASLWNWFDITNWLWYIRXFIIIVGXLIQLXIVFAVLSLV									
94UG024 DNA	.R.....DL.....I.....S.....R.....									
	710	720	730	740	750	760	770	780	790	800
94UG024 CC	NRVRQGYSPLSFQTLPTPRGPDREPGETXEEGGEQGRGRSIRLLNGLSXLIVDDLRNLCFLSYHHLRDLILIAARIVGLLGRRGWEAIKYLWNLLQYWIQ									
94UG024 DNA	.....E.....A.....									
	810	820	830	840	850					
94UG024 CC	ELKNSAISLFNTTAIVVAEGTDRIEELIQRIGRAILNIPTRIRQGLERLLL*									
94UG024 DNA	.....A.....*									



# RLUs from Tropism Assay (U87 Cells)



# Summary:

- gp160 sequence, neutralization sensitivity and tropism: good agreement between most clones and cell culture stocks
- Sequence alignments confirm matched identity for all pairs except 92BR025 vector sequence
- VI191 and 92UG024 have one or more significant differences in sensitivity to MAbs/sCD4 as well as sequence differences
- For direct comparisons between assays using the difference sources of virus exclude 92BR025, VI191 and 92UG024

# Thank you!

- Pham Phung!!!